In re Application of: Michal AMIT et al
Serial No.: 10/537,784
Filed: June 6, 2005
Office Action Mailing Date: September 15, 2008

Examiner: Deborah Crouch
Group Art Unit: 1632
Attorney Docket: 29606

In the claims:

1-152 (Cancelled).

153. (Currently amended) A method of establishing a feeder cells-free human embryonic stem cell line which is maintained in an undifferentiated, pluripotent and proliferative state, the method comprising:

(a) obtaining inner cell mass (ICM) ~~stem~~ cells from ~~of~~ a human blastocyst, ~~embryo,~~ and;

(b) culturing said ICM ~~stem~~ cells ~~of said human embryo~~ under culturing conditions devoid of feeder cells and including an extracellular matrix and a tissue culture medium supplemented with TGFβ_1 and bFGF to thereby obtain the feeder cells-free human embryonic stem cell line.

154. (Previously presented) The method of claim 153, further comprising cloning a cell from the human embryonic stem cell line resultant from step (b) under said culturing conditions.

155. (Currently amended) A method of propagating a human embryonic stem cell line in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing cells of the human embryonic stem cell line on an extracellular matrix and a tissue culture medium which comprises TGFβ_1 and bFGF to thereby maintain the cells of the human embryonic stem cell line in an undifferentiated, pluripotent and proliferative state.

156. (Currently amended) The method of claim 153, wherein said extracellular matrix is a fibronectin matrix.

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157. (Currently amended) A method of propagating a human embryonic stem cell line in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing cells of the human embryonic stem cell line on a fibronectin matrix and a tissue culture medium which comprises TGFβ_1 and bFGF, ~~The method of claim 156,~~ wherein said fibronectin is selected from the group consisting of bovine fibronectin, recombinant bovine fibronectin, human fibronectin, recombinant human fibronectin, mouse fibronectin, recombinant mouse fibronectin, and synthetic fibronectin~~.~~, to thereby maintain the cells of the human embryonic stem cell line in an undifferentiated, pluripotent and proliferative state.

158. (Currently amended) The method of claim 153, wherein said culturing conditions are ~~substantially~~ free of xeno contaminant and whereas said extracellular matrix is selected from the group consisting of human plasma fibronectin matrix, recombinant human plasma fibronectin matrix, human cellular fibronectin matrix, recombinant human cellular fibronectin matrix, synthetic fibronectin.

159. (Previously presented) The method of claim 153, wherein the human embryonic stem cell line comprises at least 85 % of undifferentiated human embryonic stem cells.

160. (Previously presented) The method of claim 153, wherein the cells of the human embryonic stem cell line maintain a doubling time of at least 25 hours.

161. (Previously presented) The method of claim 153, wherein said tissue culture medium further comprises serum and/or serum replacement.

162. (Previously presented) The method of claim 161, wherein said serum and/or said serum replacement is provided at a concentration of at least 10 %.

163. (Previously presented) The method of claim 161, wherein said serum and/or said serum replacement is provided at a concentration of 15 %.

164. (Previously presented) The method of claim 153, wherein said TGFβ_1 is provided at a concentration of at least 0.06 ng/ml.

165. (Previously presented) The method of claim 153, wherein said TGFβ_1 is provided at a concentration of 0.12 ng/ml.

166. (Previously presented) The method of claim 153, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

167. (Previously presented) The method of claim 153, wherein said bFGF is provided at a concentration of 4 ng/ml.

168. (Previously presented) The method of claim 153, wherein said tissue culture medium further comprises LIF.

169. (Previously presented) The method of claim 168, wherein said LIF is provided at a concentration of 1000 u/ml.

170. (Currently amended) A method of establishing a xeno – free, feeder cells-free mammalian embryonic stem cell line of a species which is maintained in an undifferentiated, pluripotent and proliferative state, the method comprising:

(a) obtaining inner cell mass (ICM) ~~stem~~ cells ~~of~~ from a mammalian blastocyst ~~an embryo~~ of the species, and;

(b) culturing said ICM ~~stem~~ cells under culturing conditions devoid of feeder cells ~~and xeno contaminants~~ and including a xeno-contaminant-free

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mammalian extracellular matrix derived from the same species ~~species—derived matrix devoid of xeno contaminants~~ and a tissue culture medium devoid of xeno contaminants, said tissue culture medium comprises TGFβ1 and bFGF, to thereby obtain the xeno – free, feeder cells-free mammalian embryonic stem cell line of the species.

171. (Currently amended) A method of propagating a mammalian ~~species~~ embryonic stem cell line of a species in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells and xeno contaminants, the method comprising culturing cells of the mammalian ~~species~~ embryonic stem cell line of the species on a mammalian ~~species—derived~~ extracellular matrix ~~devoid of xeno contaminants~~ of the same species and a tissue culture medium devoid of xeno contaminants, said tissue culture medium comprises TGFβ1 and bFGF, to thereby maintain the cells of the mammalian ~~species~~ embryonic stem cell line of the species in an undifferentiated, pluripotent and proliferative state.

172. (Currently amended) The method of claim 170, wherein said mammalian extracellular matrix is a ~~species—derived~~ fibronectin matrix of the same species.

173. (Currently amended) The method of claim 170, wherein said feeder cells-free culturing conditions are ~~substantially~~ free of xeno contaminants.

174. (Currently amended) The method of claim 170, wherein the mammalian ~~species~~ embryonic stem cell line of the same species comprises at least 85 % of undifferentiated ~~species~~ mammalian embryonic stem cells of the species.

175. (Currently amended) The method of claim 170, wherein the cells of the ~~species~~ mammalian embryonic stem cell line of the same species maintain a doubling time of at least 20 hours.

176. (Currently amended) The method of claim 170, wherein said tissue culture medium further comprises a ~~species-derived~~ serum derived from the same species and/or a serum replacement.

177. (Currently amended) The method of claim 176, wherein said ~~species-derived~~ serum derived from the same species is provided at a concentration of at least 5 %.

178. (Previously presented) The method of claim 176, wherein said serum replacement is provided at a concentration of at least 10 %.

179. (Previously presented) The method of claim 176, wherein said serum replacement is provided at a concentration of 15 %.

180. (Cancelled)

181. (Previously presented) The method of claim 171, wherein said tissue culture medium further comprises LIF.

182. (Previously presented) The method of claim 171, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

183. (Previously presented) The method of claim 171, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

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184. (Previously presented) The method of claim 171, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

185. (Previously presented) The method claim 171, wherein said bFGF is provided at a concentration of 4 ng/ml.

186. (Previously presented) The method of claim 181, wherein said LIF is provided at a concentration of at least 500 u/ml.

187. (Previously presented) The method of claim 181, wherein said LIF is provided at a concentration of 1000 u/ml.

188. (Currently amended) A method of establishing a xeno – free, feeder cells-free mammalian embryonic stem cell line of a species which is maintained in an undifferentiated, pluripotent and proliferative state, the method comprising:

(a) obtaining inner cell mass (ICM)~~stem~~ cells ~~of an embryo of the species~~from a mammalian blastocyst of the species, and;

(b) culturing said ICM ~~stem~~ cells under xeno-free culturing conditions devoid of feeder cells and xeno contaminants and including ~~a species – derived~~ a mammalian extracellular matrix of the same species and a ~~species derived~~ conditioned medium of the same species, to thereby obtain the xeno – free, feeder cells-free mammalian embryonic stem cell line of the species.

189. (Currently amended) A cell culture comprising undifferentiated, pluripotent and proliferative human embryonic stem cells on an extracellular matrix in a culture medium, said culture medium comprising TGFβ1 and bFGF, wherein the cell culture is ~~substantially~~ free of xeno- and feeder cells contaminants.

190. (Previously presented) The cell culture of claim 189, wherein the culture medium further comprises serum replacement.

191. (Previously presented) The cell culture of claim 190, wherein said serum replacement is provided at a concentration of at least 10 %.

192. (Previously presented) The cell culture of claim 190, wherein said serum replacement is provided at a concentration of 15 %.

193. (Previously presented) The cell culture of claim 189, wherein said culture medium further comprises LIF.

194. (Previously presented) The cell culture of claim 189, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

195. (Previously presented) The cell culture of claim 189, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

196. (Previously presented) The cell culture of claim 189, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

197. (Previously presented) The cell culture of claim 189, wherein said bFGF is provided at a concentration of 4 ng/ml.

198. (Previously presented) The cell culture of claim 193, wherein said LIF is provided at a concentration of at least 500 u/ml.

199. (Previously presented) The cell culture of claim 193, wherein said LIF is provided at a concentration of 1000 u/ml.

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200. (Previously presented) The cell culture of claim 189, wherein said human embryonic stem cells are maintainable in an undifferentiated, pluripotent and proliferative state for at least 38 passages.

201. (Previously presented) The cell culture of claim 189, wherein said human embryonic stem cells maintain a doubling time of at least 25 hours.

202. (Previously presented) The cell culture of claim 189, wherein said human embryonic stem cells comprise at least 85 % of undifferentiated stem cells.

203. (Currently amended) A xeno-free, feeder cells-free culture system comprising an extracellular matrix devoid of xeno - contaminants and a tissue culture medium devoid of xeno contaminants, said culture medium comprises TGFβ1 and bFGF, the xeno-free, feeder cells-free culture system maintains human embryonic stem cells cultured therein in a proliferative, pluripotent and undifferentiated state.

204. (Currently amended) A xeno-free, feeder cells-free culture system comprising a ~~The culture system of claim 203, wherein said matrix is~~ human-derived fibronectin matrix devoid of xeno - contaminants and a tissue culture medium devoid of xeno contaminants, said culture medium comprises TGFβ1 and bFGF, the xeno-free, feeder cells-free culture system maintains human embryonic stem cells cultured therein in a proliferative, pluripotent and undifferentiated state.

205. (Previously presented) The culture system of claim 204, wherein said human-derived fibronectin is selected from the group consisting of human plasma fibronectin, recombinant human plasma fibronectin, human cellular fibronectin, recombinant human cellular fibronectin, and synthetic fibronectin.

206. (Previously presented) The culture system of claim 203, wherein said tissue culture medium further comprises serum replacement.

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207. (Previously presented) The culture system of claim 206, wherein said serum replacement is provided at a concentration of at least 10 %.

208. (Previously presented) The culture system of claim 206, wherein said serum replacement is provided at a concentration of 15 %.

209. (Previously presented) The culture system of claim 203, wherein said tissue culture medium further comprises LIF.

210. (Previously presented) The culture system of claim 203, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

211. (Previously presented) The culture system of claim 203, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

212. (Previously presented) The culture system of claim 203, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

213. (Previously presented) The culture system of claim 203, wherein said bFGF is provided at a concentration of 4 ng/ml.

214. (Previously presented) The culture system of claim 209, wherein said LIF is provided at a concentration of at least 500 u/ml.

215. (Previously presented) The culture system of claim 209, wherein said LIF is provided at a concentration of 1000 u/ml.

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216. (Previously presented) The culture system of claim 203, wherein said human embryonic stem cells comprise at least 85 % of undifferentiated human embryonic stem cells.

217. (Previously presented) The culture system of claim 203, wherein said human embryonic stem cells maintain a doubling time of at least 25 hours.

218. (Withdrawn) A method of treating an individual in need of cell replacement and/or tissue regeneration, comprising administering a human embryonic stem cell preparation being free of xeno and feeder cells contaminants to the individual.

219. (Withdrawn and Previously presented) The method of claim 218, further comprising preparing said human embryonic stem cell preparation prior to said administering, said preparing being effected by:

(a) obtaining human embryonic stem cells, and;

(b) culturing said human embryonic stem cells under culturing conditions devoid of feeder cells and xeno contaminants and including a human-derived fibronectin matrix and a tissue culture medium supplemented with $TGF\beta_1$ and bFGF to thereby prepare the human embryonic stem cell preparation.

220. (Withdrawn) The method of claim 219, wherein said human-derived fibronectin is selected from the group consisting of human plasma fibronectin, recombinant human plasma fibronectin, human cellular fibronectin, recombinant human cellular fibronectin, and synthetic fibronectin.

221. (Currently amended) A method of maintaining human embryonic stem cells in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing the human

embryonic stem cells under culturing conditions including an extracellular matrix and a tissue culture medium, said culture medium comprises TGFβ1 and bFGF provided at a concentration range which maintains said stem cells for at least 56 passages with a doubling time of at least 25 hours.

222. (Previously presented) The method of claim 221, wherein said human embryonic stem cells comprise at least 85 % of undifferentiated human embryonic stem cells.

223. (Currently amended) A method of maintaining human embryonic stem cells in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing the human embryonic stem cells under culturing conditions including a matrix ~~The method of claim 221, wherein said matrix is~~ selected from the group consisting of human-derived fibronectin, human-derived laminin, foreskin fibroblast matrix~~,~~ and MEFs matrix~~.~~ and a tissue culture medium, said culture medium comprises TGFβ1 and bFGF provided at a concentration range which maintains said stem cells for at least 56 passages with a doubling time of at least 25 hours.

224. (Previously presented) The method of claim 223, wherein said human-derived fibronectin is selected from the group consisting of human plasma fibronectin, recombinant human plasma fibronectin, human cellular fibronectin, recombinant human cellular fibronectin, and synthetic fibronectin.

225. (Previously presented) The method of claim 221, wherein said tissue culture medium further comprises LIF.

226. (Previously presented) The method of claim 221, wherein said $TGF\beta_1$ is provided at a concentration range of 0.06-0.24 ng/ml.

227. (Previously presented) The method of claim 221, wherein said bFGF is provided at a concentration range of 2-8 ng/ml.

228. (Previously presented) The method of claim 225, wherein said LIF is provided at a concentration range of 500-2000 u/ml.

229. (Previously presented) A method of maintaining human embryonic stem cells in an undifferentiated, pluripotent and proliferative state under culturing conditions devoid of feeder cells, the method comprising culturing the human embryonic stem cells under culturing conditions including an extracellular matrix and tissue culture medium which includes serum replacement at a concentration of 15 %, $TGF\beta_1$ at a concentration of 0.12 ng/ml, LIF at a concentration of 1000 u/ml, and bFGF at a concentration of 4 ng/ml.

230. (Previously presented) The method of claim 168, wherein said LIF is provided at a concentration of at least 500 u/ml.

231. (Previously presented) The method of claim 155, wherein said $TGF\beta_1$ is provided at a concentration of at least 0.06 ng/ml.

232. (Previously presented) The method of claim 155, wherein said $TGF\beta_1$ is provided at a concentration of 0.12 ng/ml.

233. (Previously presented) The method of claim 155, wherein said bFGF is provided at a concentration of at least 2 ng/ml.

234. (Previously presented) The method of claim 155, wherein said bFGF is provided at a concentration of 4 ng/ml.

235. (Previously presented) The method of claim 155, wherein said tissue culture medium is further supplemented with LIF.

236. (Previously presented) The method of claim 235, wherein said LIF is provided at a concentration of at least 500 u/ml.

237. (Previously presented) The method of claim 235, wherein said LIF is provided at a concentration of at least 1000 u/ml.